Int. J. Exp. Path. (1991) 72, 437-450 ADONIS 0959967391000403

Electron microscopy of rat lung following a single acute exposure to perfluoroisobutylene (PFIB). A sequential study of the first 24 hours following exposure

R.F.R. Brown and P. Rice
Pathology and Clinical Toxicology Section, Chemical and Biological Defence Establishment, Porton Down, Salisbury, Wilts, UK

Received for publication 1 October 1990
Accepted for publication 19 March 1991

Summary. The histopathology of rat lung has been studied after an acute exposure to perfluoroisobutylene (PFIB) at a concentration of 638 mg/m^3 (78 p.p.m.) for 1.5 min giving a Ct = 957 mg min/m^3 for the first 24 h following exposure. Within 5 min of exposure changes to the bronchioles and peribronchial alveoli were observed which took the form of alterations to cilial structure, increased pinocytosis and electron lucency, with occasional vesicle formation of type 1 alveolar epithelial cells. Intercellular leakage with minimal fluid accumulation in the alveolar spaces was also seen. The very rapid action of PFIB strongly suggests a direct action by the compound. There then followed the gradual development of pulmonary oedema which was visible histologically 2-3 h post exposure with deaths occurring from 7 h onwards. Animals sacrificed at 24 h post exposure showed evidence of widespread pulmonary oedema and alveolar interstitial infiltration by lympho-mononuclear cells and macrophages.

Keywords: perfluoroisobutylene, PFIB, pentafluoro-(2-trifluoromethyl)-propene, perfluoroisobutene, pulmonary oedema, electron microscopy, histopathology

Pentafluoro-(2-trifluoromethyl)-propene; perfluoroisobutene; perfluoroisobutylene; (PFIB) is a potent lung oedemagen, whose toxicological effects, chemical and physical properties have been reviewed elsewhere (Zeifman 1984; Clayton 1977). PFIB has been implicated as one of the products produced during fires by the pyrolysis of polymers such as polytetrafluoroethylene and perfluoroethylpropylene (Waritz & Kwou 1968). These compounds produce symptoms of intoxication which include nausea, shivering, fever, sore throat, cough, weakness and a sense of oppression and tightness in the chest. Pulmonary oedema has also been reported (Karpov 1977).

Makulova (1965) reported five human cases of acute PFIB exposure with one fatality. He noted symptoms which included headache, cough, substernal pain, dyspnoea and fever all intensifying within the first hour following exposure and concluded that

Correspondence: Dr R.F.R. Brown, Pathology and Clinical Toxicology Section, Medical Division, Procurement Executive of Defence, Chemical and Biological Defence Establishment, Porton Down, Salisbury, Wilts SP4 0JQ, UK.

PFIB acted in the lower, rather than the upper, respiratory tract, causing pneumonia accompanied by pulmonary oedema.

In animal studies the phenomenon of 'lightning death' has been reported by Karpov (1977) with collapse and death occurring within 1 min of exposure to high concentrations of PFIB. This is in contrast to the normally delayed deaths, due to pulmonary oedema, which have been reported after a wide variety of gaseous toxic insults (Terplitz 1979). In studies of rats exposed to PFIB, the 0.25-min LCt_{50} was determined as 361 p.p.m. (2953 mg/m^3) while the 10-min LCt_{50} was 17 p.p.m. (139 mg/m^3). The relation between concentration and mortality was constant over this 0.25–10-min time period. Affected rats either died with gross pathological signs of pulmonary congestion or recovered with no apparent residual effects (Smith *et al.* 1982).

In this present investigation we have studied the changes in the structure of rat lung using both light and electron microscopy following a single exposure to PFIB. By selecting suitable time intervals, we have observed the transient changes leading to the onset of pulmonary oedema.

Materials and methods

Materials and animals

PFIB was synthesized by Organic Chemistry Section, Chemical Defence Establishment, Porton Down, Salisbury, UK with a purity of 96%, the residue comprising mainly fluoropropane. Glutaraldehyde and the components of Spurr's or TAAB resin were supplied by TAAB Laboratories Equipment Ltd, Reading, Berkshire, UK and all other chemicals were obtained from British Drug Houses PLC, Poole, Dorset, UK. Female Porton Strain Wistar derived rats were obtained from the Animal Breeding Unit, Porton Down, Salisbury, UK, wt 200–230 g. They were kept at an ambient temperature of 20°C and humidity (40–60%) and fed on a Porton Combined Diet obtained from Special Diet Services, Manea, Cambs, UK.

Exposure

Fifty rats, randomized into groups of five, were exposed, head only, to PFIB. They were exposed in groups of 10 using a small rectangular (12-l capacity) exposure chamber containing side ports into which the rats, constrained within metal cylinders with their heads stocked, could be inserted. The stocked head provided a seal, such as to give a head only exposure. PFIB was metered at approximately 10 ml/min from a gas cylinder by way of a flowmeter (range 4–20 ml/min) into the exposure chamber. Before and after the exposure, a pump was used to divert the flow of material away from the exposure chamber. The exposure time was 1.5 min. The flow through the exposure chamber was 140 l/min and all the effluent was monitored for PFIB using a MIRAN infrared gas analyser set at a wavelength of 8.4 μm. The concentration of PFIB in the exposure chamber was 638 mg/m^3 (78 p.p.m.) giving a total Ct of 957 mg min/m^3. This Ct is probably near to a LCt_{100}, the head only LCt_{55} for PFIB being approximately 750 mg min/m^3 (J.A.G. Edginton & P.N. Price 1985, unpublished).

The animals were caged in groups of five, monitored continuously, and any toxic signs noted throughout the whole course of the experiment. They were then killed, in groups of five, either within 5 min of exposure or at 1, 2, 3, 4, 6, 8, 12, 16 or 24 h following exposure. A control group consisting of five animals were sham exposed to air only, maintained in the same conditions as the PFIB-exposed animals, and killed at 24 h after exposure.

Autopsy and tissue selection

The animals were killed by intraperitoneal injection of Sagatal (sodium pentobarbitone 60 mg/ml). The thorax was opened and the lungs rapidly removed and immersed in

3% glutaraldehyde in 0.1M cacodylate buffer pH 7.4 containing 3 mM calcium chloride which had been cooled to 4°C for a few minutes. Preliminary experiments had established that this method of fixation was preferable to monitor oedema as recommended by others (Dungworth *et al.* 1976) although it has the disadvantage that the structural morphology of the lung is not as well preserved as with perfusion fixation. Samples for electron microscopy were taken in the form of a 3-mm thick paramedian transverse slice from the left and lower right lobes and placed in glutaraldehyde fixative which had been cooled to 4°C for a further 24 h. The remaining lobes of the lung together with samples of larynx, trachea, heart, thymus, liver, spleen, adrenals, kidney and small intestine were placed in 10% neutral buffered formalin for light microscopy.

Light microscopy

Paramedian transverse slices were taken from the left lung and the upper and lower lobes of the right lung and after further fixation in 10% neutral buffered formalin were dehydrated and embedded in paraffin wax. Sections, 5 ± 2 μm thick, were cut and stained with haematoxylin and eosin using a Histostainer (Miles Laboratories Ltd, Stoke Poges, Berkshire, UK). The concentration of eosin was increased from 0.1% dissolved in 95% ethanol to 0.5% in order to enhance the staining of the eosinophilic components of intra-alveolar oedema. All other organs were treated similarly except that they were stained using the Histostainer with 0.1% eosin in 95% ethanol.

Electron microscopy

Electron microscopic examination was carried out only on the lungs of controls and those animals which had been killed at 5 min, 1 h, 3 h, 6 h and 24 h after exposure to PFIB. After fixation for 30 min the 3-mm slices were diced into approximately 1 mm × 1 mm cubes and further fixed in glutaraldehyde for at least 8 h. Subsequent dehydration and infiltration in either Spurr's low viscosity resin or TAAB resin was carried out using standard techniques utilizing a Lynx processor (Leica UK Ltd, Milton Keynes, UK). Polymerization was carried out at 70°C for between 12 and 24 h. At least five blocks from each lobe were cut on an Ultracut E (Leica UK Ltd, Milton Keynes, UK) ultramicrotome using both glass and diamond knives. 1-μm thick sections were cut for light microscopy and stained for 20 min at 60°C with 1% Toluidine blue in 1% sodium tetraborate. Sections were then cut for electron microscopy, 60–90 nm thick, picked up on copper grids and stained with alkaline lead citrate using an Ultrostainer (Leica UK Ltd, Milton Keynes, UK). They were then examined and micrographs taken using a Phillips EM300 electron microscope operated at 80 kV.

Results

Physical signs

For the first 5 h following exposure, the animals showed little sign of intoxication. After this time the animals became progressively lethargic with piloerection, unsteady gait and stertorous respirations. Two animals in the 8-h group were humanely killed *in extremis* at approximately 7 h, none of the 12-h group survived beyond 9.5 h whilst the 16-h group were humanely killed between 10 and 16 h. Only three of the 24-h group survived to be killed at the designated time. The dying showed signs of collapse, terminal anoxia and convulsions at which time they were humanely killed. At this time a frothy serous fluid exuded from the mouth and nose. The survivors from groups killed later than 6 h showed similar but less severe signs to those described above, with only slight serous exudate around the mouth and nose. The three survivors from the 24-h group appeared near normal at the time when they were killed.

Macroscopic appearances

The lungs of rats exposed to PFIB showed isolated incidences of patchy congestion, pale discolouration and petechial haemorrhages. Froth exuded from the cut surfaces of the lungs of animals which were autopsied at times later than 4 h after exposure. No abnormalities were detected in any of the other organs which were examined.

Light microscopy

The structure of the normal Porton strain rat lung has been described previously (Colgrave *et al.* 1979). No significant alterations in lung structure from those described could be detected in the controls except for isolated foci of peribronchial or perivascular lymphocytic infiltration and a generalized parenchymal collapse which is characteristic of the immersion method of fixation. No abnormalities were observed in sections from any other organs which were taken at autopsy. In animals which were exposed to PFIB, histological changes were observed only in the lung with no significant pathological abnormality being observed in any other organ.

At 1 h following exposure the lungs of all animals showed focal perivascular infiltrates of eosinophils and a more generalized mild alveolar capillary congestion. By 2 and 3 h following exposure, a mild to moderate perivascular oedema with oedema fluid containing a mainly eosinophil cellular infiltrate was observed in all animals. Focal acute inflammatory cell infiltration, which extended into the lung parenchyma, was also evident. Associated with these changes were areas of focal intra-alveolar fibrin deposition. A more generalized moderate alveolar capillary congestion was seen. In animals killed at 4 h following exposure similar features to those observed in the previous groups were seen in all animals. Additionally, 2/5 animals showed focal intra-alveolar oedema, intra-alveolar fibrin, and a generalized acute alveolitis with abundant polymorphonuclear leucocytes. All animals killed between 6 and 12 h following exposure showed a moderate to severe interstitial, perivascular and intra-alveolar oedema. Evidence of diffuse, acute alveolitis was observed together with focal accumulations of intra-alveolar foamy macrophages. Generalized moderate alveolar capillary congestion was also seen. In animals killed at 16 h following exposure, severe generalized interstitial and intra-alveolar oedema was again a dominant feature. There was a persistent alveolitis, but with the abundant polymorphonuclear leucocytes beginning to be replaced by a parenchymal infiltrate of mononuclear cells. There were widespread intra-alveolar macrophage accumulations together with focal areas of hypoxic dilatation of alveolar spaces. One animal showed focal type II pneumocyte hyperplasia. By 24 h following exposure 4/5 animals showed similar features to those described in animals which were killed at 16 h following exposure. Observation of the lung from the remaining animal showed a diminished intra-alveolar oedema with diffuse alveolar septal consolidation with large macrophages and occasional lymphocytes. Focal type II pneumocyte hyperplasia was also seen.

Electron microscopy

The ultrastructure of the normal Porton rat lung has been described previously (Colgrave *et al.* 1979). In the lungs of control animals the only difference from that already described was isolated incidences of increases in perivascular or peribronchiolar cells, particularly lymphocytes. Alveolar capillary changes took the form of very isolated endothelial 'ballooning' which is compatible with stress-related changes previously described (Colgrave *et al.* 1979).

The changes observed following exposure to PFIB were confined mainly to the alveoli, bronchioles and alveolar vasculature. Only animals which survived to their designated time of sacrifice were utilized in this investigation.

Bronchiolus

Animals killed within 5 min of exposure to PFIB (n=5)

All animals showed crenulation of the ciliary plasma membrane from ciliated bronchiolar epithelial cells together with loss of cytoplasmic matrix and structure as evidenced by increased cytoplasmic lucency (Fig. 1). Proteinaceous fluid, as denoted by traces of electron-dense amorphous material, was also present in the bronchiolar lumen in 3/5 animals examined.

Animals killed 1 h after exposure (n=5)

The alterations seen were similar to those in the previous group except that cilial changes appeared to be more widespread with the first signs of the necrotic process in ciliated epithelial and Clara cells of the bronchioles in 2/5 animals. This took the form of mitochrondial swelling with perinuclear vacuolation.

Animals killed 3 h after exposure (n=5)

The alterations observed were similar to those found at 1 h post exposure with necrosis of the bronchiolar epithelium being found in the lungs of 3/5 animals (Fig. 2).

Animals killed 6 h after exposure (n=5)

The lungs of all animals showed severe and more generalized alterations to the cells of the bronchiolar epithelium leading to total disruption and sloughing of the epithelium in 2/5 animals. Evidence of accumulation of fluid together with macrophages and haemorrhage was observed in 3/5 animals.

Animals killed 24 h after exposure (n=3)

The changes observed were similar to those found in the previous group with signs of bronchiolar epithelial cell necrosis and fluid in the bronchiolar lumen being observed in all animals.

The alveolus

Animals killed within 5 min of exposure to PFIB (n=5)

Alveolar changes were observed only in those alveoli adjacent to the bronchioles and took the form of increased pinocytosis and very isolated vesication of type 1 alveolar epithelial cells. Leakage of electron-dense fluid occurred into the alveolar space apparently from widening of the intercellular tight junctions (Fig. 3). Minor accumulations of electron-dense amorphous fluid were seen in a few alveolar spaces. A generalized loss of the structure of the laminations from lamellated bodies of Type II pneumocytes was seen in 4/5 animals.

Animals killed 1 h after exposure (n=5)

As with the previous group, the changes were observed mainly in peribronchiolar alveoli and consisted of increased type 1 alveolar epithelial cell pinocytosis, vesicles (these appeared to be an extreme enlargement of pinocytotic vesicles and were bounded by a unit membrane (Fig. 4)) with isolated incidences of 'blebbing' of the cell into the alveolar space. Occasional interstitial oedema was present in all animals from this group. The incidence of intra-alveolar oedema and changes to type II pneumocytes were similar to those found in the previous group. One animal showed isolated type II pneumocyte necrosis.

Animals killed 3 h after exposure (n=5)

The changes seen were similar to those described at 1 h post exposure but with an increased severity and more generalized distribution. Isolated incidences of type 1 epithelial cell necrosis, as exemplified by plasmalemmal disruption, increased cytoplasmic electron lucency, and disruption of the nucleus was observed in the lungs of 3/5 animals. Both interstitial and intra-alveolar oedema had a more generalized distribution

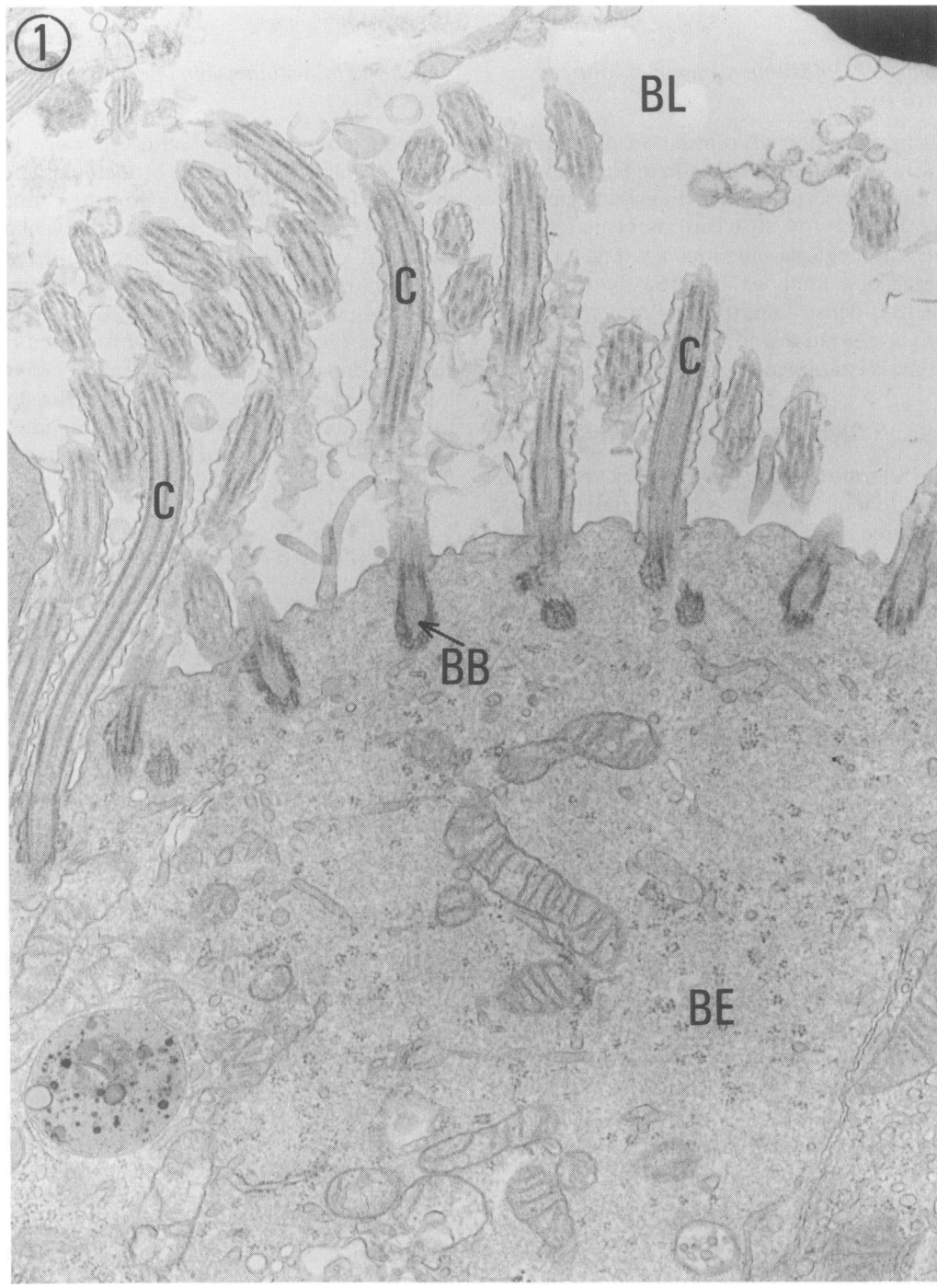

Fig. 1. Electron micrograph of the bronchiolar epithelium from the lung of rat exposed to perfluoroisobutylene and killed within 5 min of exposure. There is loss of cytoplasmic matrix and structure of the cilia as evidenced by increased cytoplasmic lucency together with an increased invagination of the plasmalemma. BL, Lumen of bronchiolus; BE, bronchiolar epithelium; C, cilia; BB, cilial basal body. × 21 375.

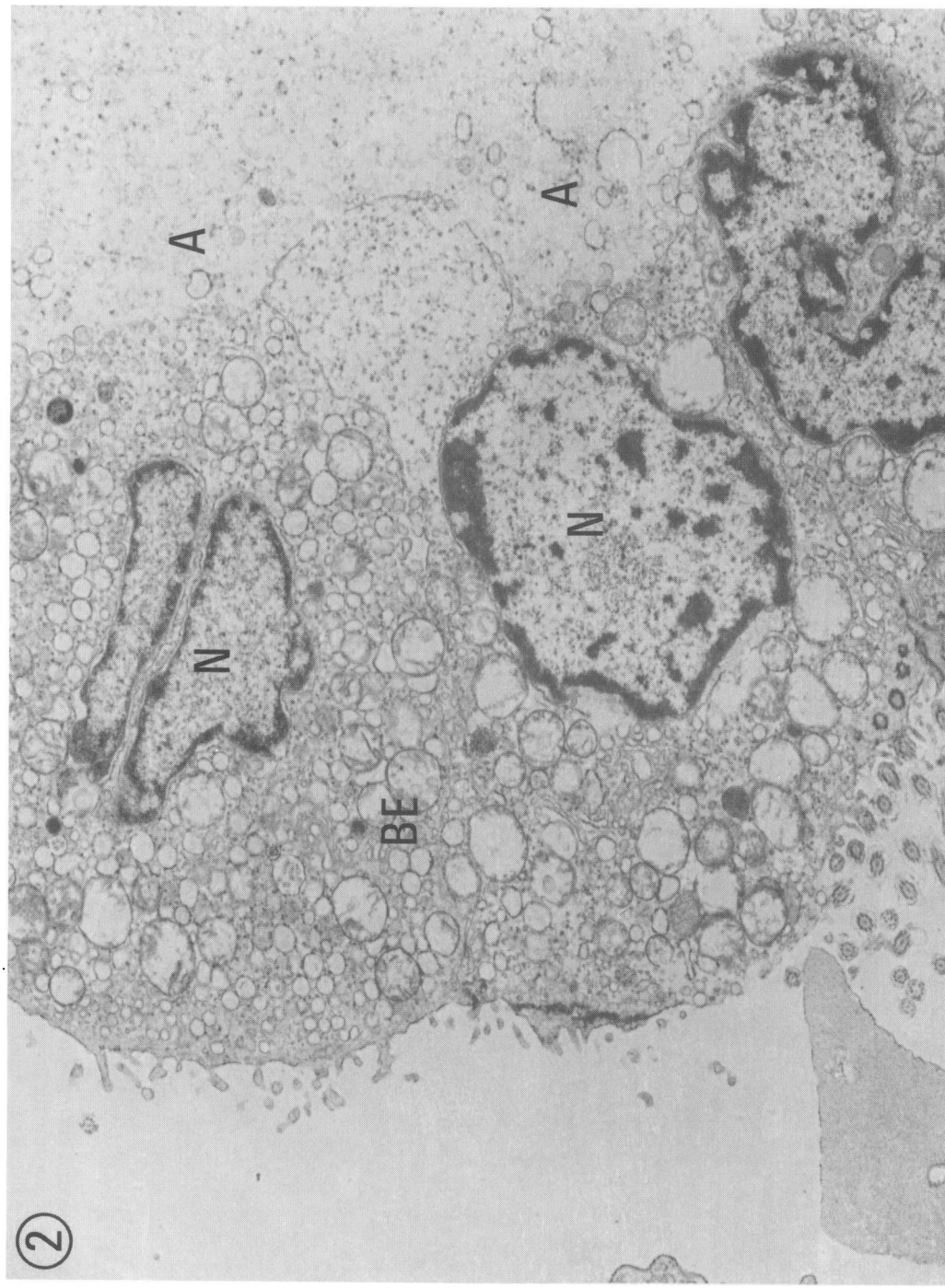

Fig. 2. Electron micrograph of the bronchiolar epithelium from the lung of rat exposed to perfluoroisobutylene and killed 3 h after exposure. A, Area of separation from the basement membrane; BE, bronchiolar epithelium showing signs of advanced necrotic change; N, nucleus. ×9550.

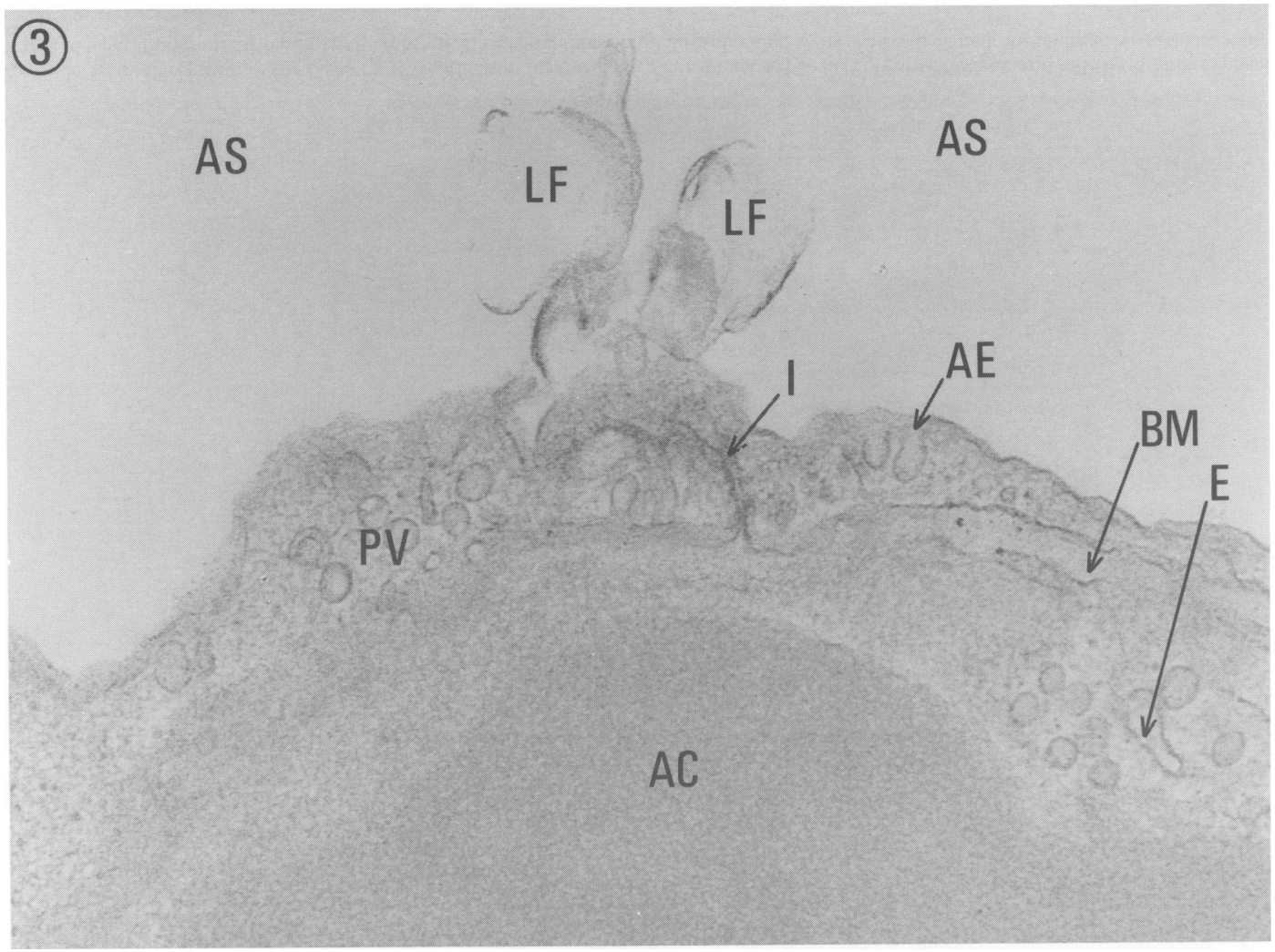

Fig. 3. Electron micrograph of the alveolus from the lung of rat exposed to perfluoroisobutylene and killed within 5 min of exposure. There is leakage of fluid into the alveolar space from the type 1 epithelial cell junction which appears widened. AC, Alveolar capillary; AS, alveolar space; AE, type 1 alveolar epithelial cell; BM, basement membrane; E, endothelium; I, intracellular junction; LF, leakage of fluid; PV, pinocytotic vesicles. × 54 675.

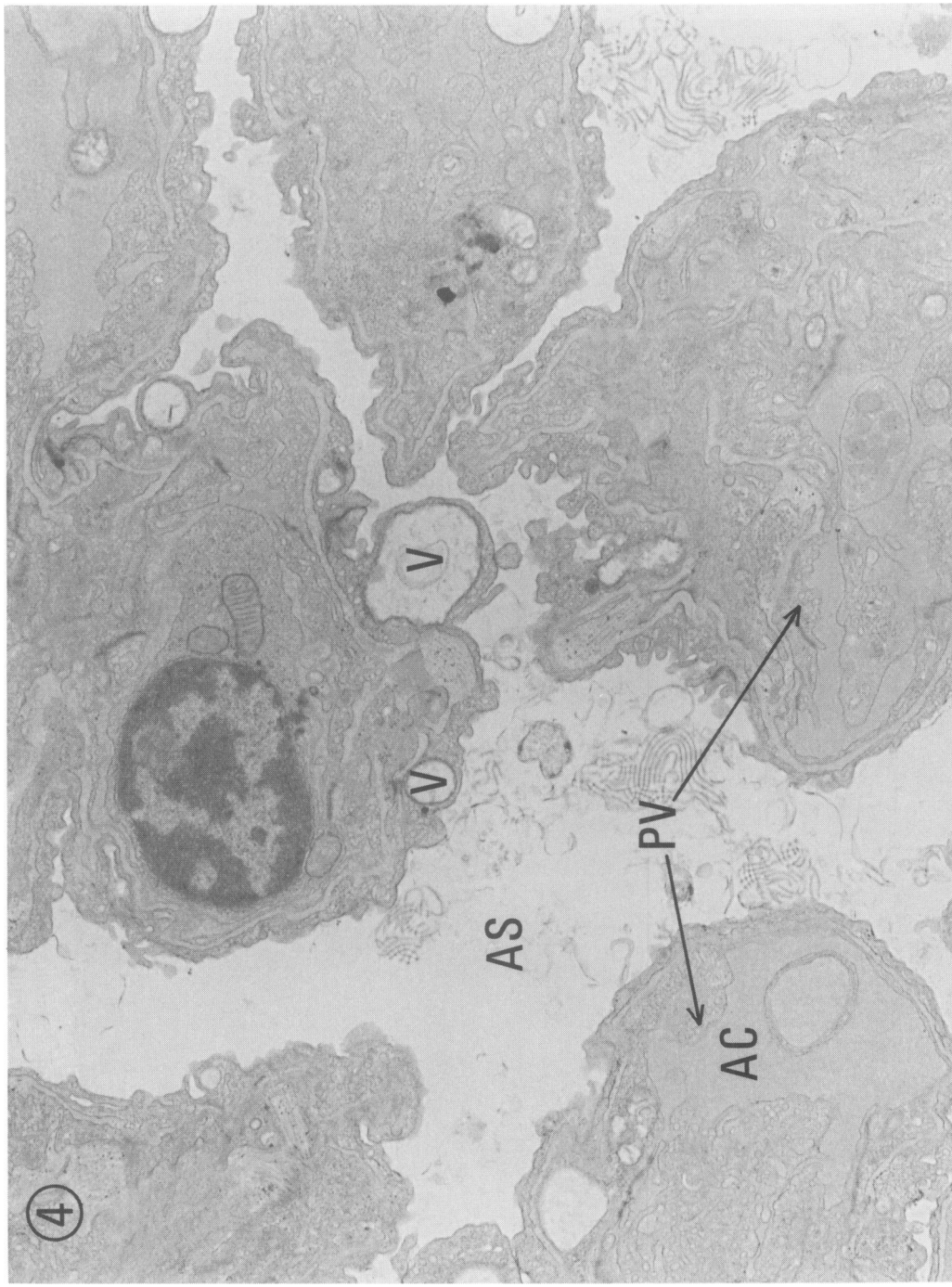

Fig. 4. Electron micrograph of alveolus from the lung of rat exposed to perfluoroisobutylene and killed 1 h after exposure. AC, Alveolar capillary; AS, alveolar space; PV, increased pinocytosis of both endothelium and type 1 epithelium; V, enlarged vesicles of the type 1 epithelium. × 11 630.

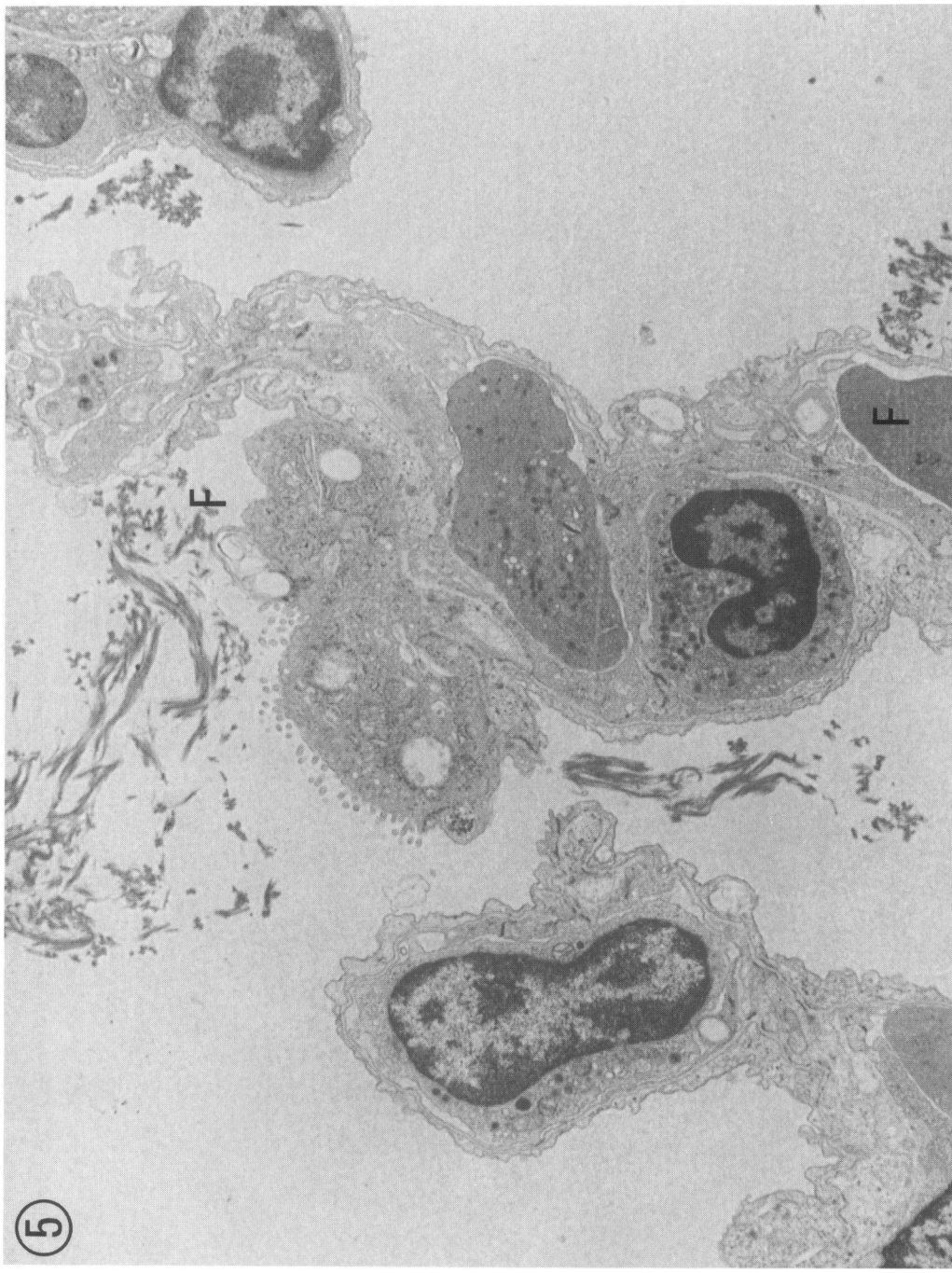

Fig. 5. Electron micrograph of alveolus from the lung of rat exposed to perfluoroisobutylene and killed 6 h after exposure. Area of gross intra-alveolar oedema with fibrin accumulations. F, Fibrin ×5450.

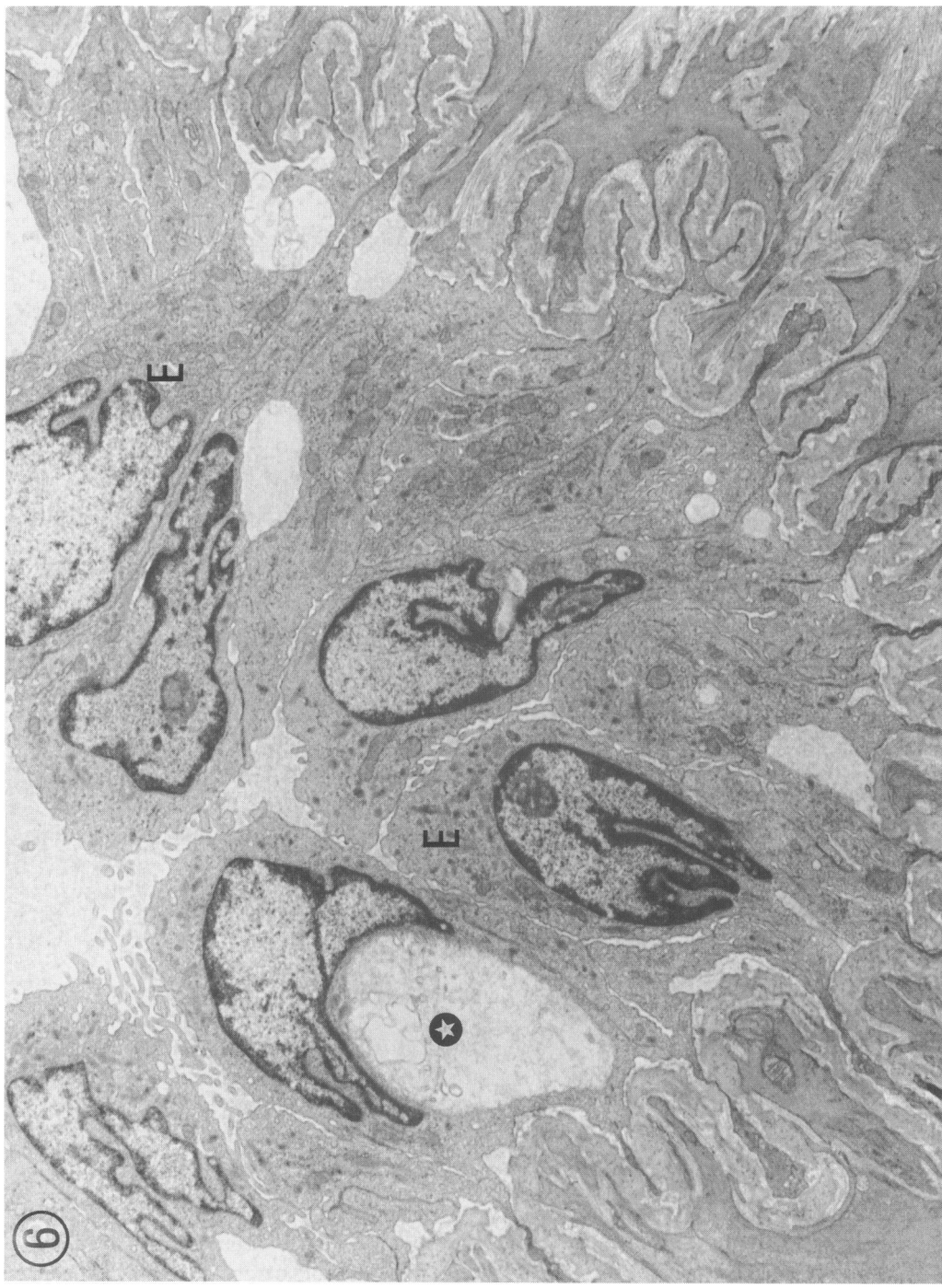

Fig. 6. Electron micrograph of arteriole from the lung of rat exposed to perfluoroisobutylene and killed 6 h after exposure. E, Arteriolar endothelium. * Perinuclear vacuolation. × 7900.

with an increased severity. Intra-alveolar cells, mainly macrophages, were slightly increased in 2/5 animals.

Animals killed 6 h after exposure (n=5)

The most obvious finding was widespread and severe intra-alveolar oedema with clots of fibrin evident in the alveolar spaces. Associated with these features was a generalized necrosis of type 1 alveolar epithelial cells with 'blebbing' of the cytoplasm into the alveolar space together with early pyknotic changes (Fig. 5). Isolated necrosis of type II pneumocytes was observed. Interstitial oedema appeared again more severe and widespread than that observed at 1 h post exposure to PFIB. The lungs of all animals showed an increase of intra-alveolar cells particularly macrophages and leucocytes.

Animals killed 24 h after exposure (n=3)

Intra-alveolar and interstitial oedema was of a similar severity to that seen previously with the fibrin clots more abundant and numerous macrophages with the occasional leucocyte being found in the alveolar spaces. Increased numbers of macrophages, monocytes and leucocytes were also found in the interstitium, being most prominent in the perivascular interstitium but also observed in the inter-alveolar interstitium. Isolated incidences of Type II pneumocyte hyperplasia were also seen in the lungs of two animals.

Microvasculature

Animals killed within 5 min of exposure to PFIB (n=5)

Increased incidences of 'ballooning' of endothelial cells into the alveolar capillary lumen, as compared to that observed in the control animals, were seen in the lungs of all animals. The endothelium of both venules and arterioles appeared normal but aggregations of platelets were observed in 2/5 animals.

Animals killed 1 h after exposure (n=5)

The incidence and severity of endothelial 'ballooning' appeared slightly increased as compared to the 5-min group whereas little change to either arteriolar or venular endothelial cells could be determined except for an isolated area of platelet aggregation.

Animals killed 3 h after exposure (n=5)

The alveolar capillary endothelium appeared to be less severely affected as compared to that seen at 1 h post exposure, but 'ballooning' of the endothelium of both arterioles and venules was observed in 2/5 animals.

Animals killed 6 h after exposure (n=5)

Alveolar capillary changes appeared similar to those observed 3 h after exposure. However, perinuclear vacuolation of both arteriolar and venular endothelia was observed in 3/5 animals (Fig. 6).

Animals killed 24 h after exposure (n=3)

No difference, as compared to controls, could be seen in animals from this group. Two animals showed increased polymorphonuclear leucocytes in the lumen of both venules and arterioles.

Discussion

The toxicology of the fluoroalkenes, of which PFIB is a member, has been extensively reviewed (Clayton 1977). PFIB is a potent lung toxicant whose main effect results in the development of pulmonary oedema with death of the affected animals approximately 1 day after inhalation. This was confirmed by the present experiment where animals died at between 8 and 24 h post exposure with histological evidence of pulmonary oedema. Other members of the group, such as hexafluoropropylene and chlorotrifluoroethylene, also cause lung injury and at lower concen-

trations cause dose-dependent damage in the kidney. In this study there was no histological evidence of renal damage. Karpov (1977) reported later pathological changes to the lung in survivors following PFIB exposure and observed symptoms of focal and lobar pneumonia at between 3 and 12 days. At 6–9 months following exposure a histological change described as 'pneumosclerosis' in English translation was seen in a few animals. The meaning of the term—pneumosclerosis—is, however, uncertain.

The pathological and electron microscopical observations of the changes leading to pulmonary oedema following PFIB inhalation were generally similar to other types of chemically induced pulmonary oedema, for example ozone, mixed oxides of nitrogen or high concentrations of oxygen (Witschi & Cote 1977; Brown *et al.* 1983; Smith 1983). There does not appear, however, to be a latent period with PFIB, in the sense that there is no period after exposure where apparent damage to the cells of the lung could not be detected. The first signs of damage, which were observed within 5 min of a 1.5-min exposure, were alterations to the structure of the plasmalemmal membrane and cytoplasm of cilia from ciliated bronchiolar epithelial cells. This was accompanied by changes to peribronchiolar alveoli which took the form of increased type 1 epithelial cell pinocytosis, isolated vesicle formation and intercellular leakage from tight junctions. These changes have also been reported following exposure to high concentrations of oxygen using morphological techniques (Barry & Crapo 1985), and would appear to be the result of a direct action of PFIB on the cells. Cilial damage, which was similar to, but more extensive than, that observed in the present experiment has been reported following a 7-month exposure to low concentrations of NO_2 (Yamamoto & Takahashi 1984).

More generalized effects include the loss of lamellar bodies from type II pneumocytes which may represent attempts by the lung to replace altered or damaged surfactant. Direct chemical attack on surfactant is thought to be unusual although vulnerable moieties are present in the composition of surfactant (Harwood & Richards 1985). Aggregations of platelets, which were observed up to 1 h after exposure in all components of the microvasculature, can lead to mediator release, vasoconstriction, attraction and sequestration of inflammatory cells, tissue damage and oedema. It is thought to be mainly a secondary phenomenon and a result of cell membrane damage (O'Flaherty 1983). However, in pulmonary oedema resulting from haemorrhagic shock, platelet aggregations may play a primary role in oedema formation (Wilson 1972).

There then followed the gradual development of frank pulmonary oedema with animals dying at approximately 7 h after exposure. Intra-alveolar oedema, as visualized using light microscopy, was evident at 3–4 h following exposure and its development was accompanied by type 1 epithelial cell necrosis, interstitial oedema, and only isolated damage to the microvascular endothelial cells.

At 24 h post exposure, ultrastructural examination revealed areas of bronchiolar epithelial necrosis and sloughing, and generalized pulmonary oedema with numerous intra-alveolar macrophages and occasional polymorphonuclear leucocytes. Interstitial lymphocytes and macrophages appeared increased and were generally distributed. Isolated type II pneumocyte hyperplasia was also present. These features are indicative of the onset of reparative processes which could possibly progress to fibrogenesis and ultimately fibrosis such as that which occurs following lung injury by paraquat (Dunnill 1982). Necrosis and sloughing of the bronchiolar epithelium may repair normally or progress to obliterating bronchiolitis by an influx of collagen and reticulin into the bronchiolar lumen. This has been observed following human exposure to NO_x (Horvath *et al.* 1978) and also in chronic myeloid leukaemia patients treated with busulphan (Kirschner & Esterly 1971).

References

BARRY B.E. & CRAPO J.D. (1985) Application of morphometric methods to study diffuse and focal injury caused by toxic agents. *CRC Crit. Rev. Toxicol.* **14**, 1–43.

BROWN R.F.R., CLIFFORD W.E., MARRS T.C. & COX R.A. (1983) The histopathology of rat lung following short term exposures to mixed oxides of nitrogen [NO_x]. *Br. J. Exp. Path.* **64**, 579–583.

CLAYTON J.W. (1977) Toxicology of the fluoroalkenes: review and research needs. *Environ Hlth Persp.* **21**, 255–267.

COLGRAVE H.F., BROWN R.J.R. & COX R.A. (1979) Ultrastructure of rat lungs following exposure to aerosols of dibenzoxazepine [CR]. *Br. J. Exp. Path.* **60**, 130–141.

DUNGWORTH D.L., SCHWARTZ L.W., TYLER W.S. & PHALEN R.F. (1976) Morphological methods for evaluation of pulmonary toxicity in animals. *Ann. Rev. Pharmacol. Toxicol.* **16**, 381–399.

DUNNILL M.S. (1982) *Pulmonary Pathology*. Edinburgh: Churchill, Livingstone. pp. 227–239.

HARWOOD J.L. & RICHARDS R.J. (1985) Lung surfactant. *Mol. Aspects Med.* **8**, 423–514.

HORVATH E.P., DOPICO G.A., BARBEE R.A. & DICKIE H.A. (1978) Nitrogen dioxide-induced pulmonary disease. *J. Occupat. Med.* **20**, 103–110.

KARPOV B.D. (1977) Establishment of upper and lower toxicity parameters of perfluoroisobutylene toxicity. *Tr. Lenig. Sanit-Gig. Med. Inst.* **111**, 30–33.

KIRSCHNER R.H. & ESTERLY J.R. (1971) Pulmonary lesions associated with Busulphan therapy of chronic myelogenous leukaemia. *Cancer* **27**, 1074–1080.

MAKULOVA I.D. (1965) Clinical picture of acute poisoning with perfluoroisobutylene. *Gig. Tr. Prof. Zabol.* **9**, 20–23.

O'FLAHERTY J.T. (1983) Lipid mediators of acute allergic and inflammatory reactions. In *Inflammatory cells and disease*. Ed. W.S. Lynn. CRC Press, Florida. pp. 84–106.

SMITH L.J. (1983) The effect of methylprednisolone on lung injury in mice. Prevention of Type 1 cell and endothelial cell damage. *J. Lab. Clin. Med.* **101**, 629–635.

SMITH L.W., GARDNER R.J. & KENNEDY G.L. (1982) Short term inhalation toxicity of Perfluoroisobutylene. *Drug Chem. Toxicol.* **5**, 295–304.

TERPLITZ C. (1979) Pulmonary cellular and interstitial oedema. In *Pulmonary Oedema*. Eds A.P. Fishman & E.M. Renkin. American Physiological Society, pp. 97–112.

WARITZ R.S. & KWOU B.K. (1968) The inhalation toxicity of pyrolysis products of polytetrafluorethylene heated below 500°C. *Am. Ind. Hyg. Assoc. J.* **29**, 19–29.

WILSON J.N. (1972) Treatment and prevention of pulmonary cellular damage with pharmacologic doses of corticosteroid. *Surg. Gyn. Obstet.* **134**, 675–681.

WITSCHI H.P. & COTE M.G. (1977) Primary pulmonary response to toxic agents. *CRC Crit. Rev. Toxicol.* **5**, 23–30.

YAMAMOTO I. & TAKAHASHI M. (1984) Ultrastructural observations of rat lung exposed to nitrogen dioxide for 7 months. *Kitasato Arch. Exp. Med.* **57**, 57–65.

ZEIFMAN Y.V., TERGABRIELYAN E.G., GAMBARYAN N.P. & KNUNYANTS I.L. (1984) Chemistry of perfluoroisobutylene. *Uspekhi-Khmii* **53**, 431–461.